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The effect of macrofaunal disturbance on *Cerastoderma edule* post-larvae.

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Abstract

Populations of the Common European cockle (*Cerastoderma edule*) often have highly patchy distributions and variable recruitment success. One of the proposed reasons is that high densities of filter feeders and / or bioturbators are thought to reduce the success of larval settlement and post-settlement survival, but the direct causal processes driving these observations are not clearly identified and validated. Through combined field and laboratory experiments, we test the hypothesis that macrofauna cause decreases in post-larval density through feeding and movement activities. The effect of excluding the bioturbating lugworm *Arenicola marina* and filter-feeding adult cockles on post-larval cockle densities was estimated in separate field experiments at two locations from the time of initial larval settlement in May 2012 to late summer August 2012. Lugworm exclusion led to a significant increase in cockle post-larval densities whereas the opposite was true for adult cockles, where exclusion led to a reduction in *C. edule* post-larval density. Although clear effects were observed in the field, experiments conducted in the laboratory failed to detect changes in mortality or byssus drifting of post-larvae as a consequence of macrofaunal activity. This study demonstrates that the presence of macrofauna can have both positive and negative effects on post-settlement density of *C. edule* post-larvae. Thus the density, distribution and identity of macrofauna have significant effects on the density and spatial distribution of *C. edule* post-larvae during the post-settlement period. These observations have implications for conservation and fishery management of this species.

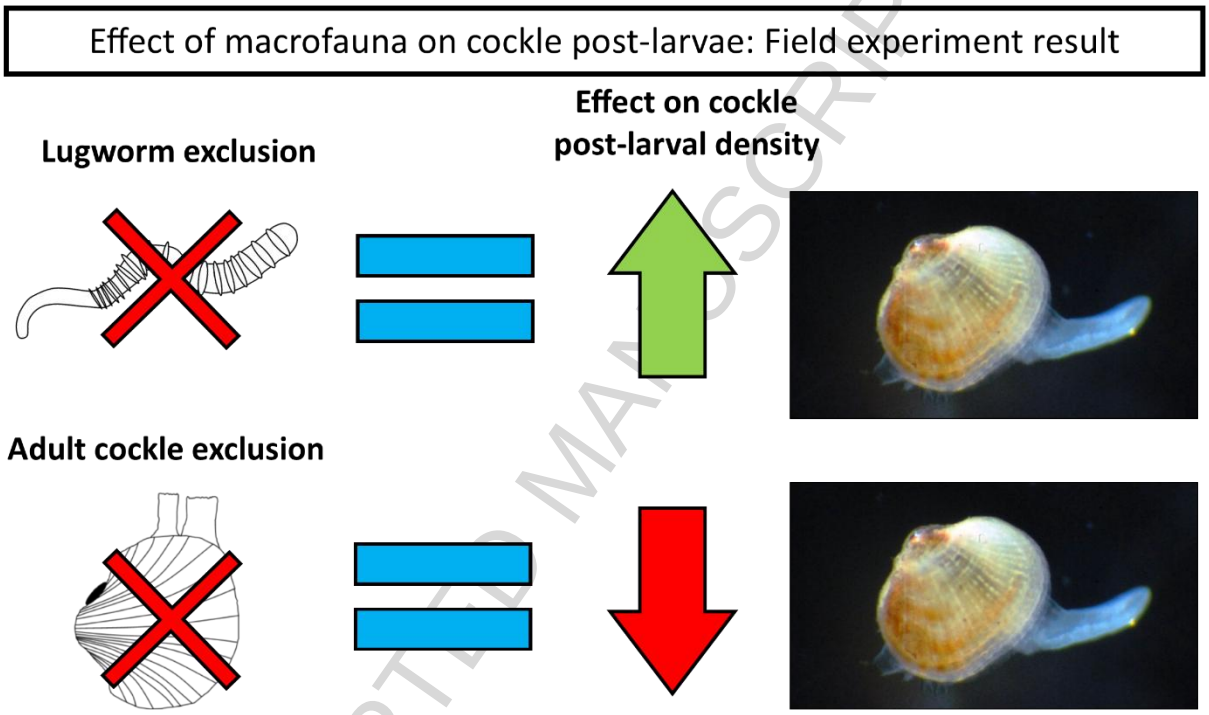
Keywords: *Arenicola marina, Cerastoderma edule*, interaction, post-larvae, cockle, settlement

Highlights

- The effect of macrofauna presence on colonisation of cockle post-larvae was tested.
- Combined field and laboratory studies were used.

- Macrofauna affected cockle post-larval density after settlement in the field study.
- The exclusion of lugworm increased cockle post-larval density in the field.
- The exclusion of adult cockles decreased cockle post-larval density in the field.

Graphical Abstract



1. Introduction

Identifying processes influencing the success and spatial distribution of recruitment in populations is important for our understanding of ecosystems (Sutherland et al., 2013). Ascertaining and disentangling key processes driving recruitment in intertidal soft sediment systems is challenging due to the highly mobile early life stages of many species, and the importance of biological interactions (Reise, 2002; Rhoads and Young, 1970). The common European cockle *Cerastoderma edule* is an important species on North East Atlantic soft-sediment tidal flats, both commercially (Hicken, 2008) and ecologically (Flach, 1996). The cohort strength and distribution of adults is largely determined during the post-settlement stage (0-group), which is considered as the period from larval settlement through to survival of their first winter (Beukema and Dekker, 2005). The occurrence of high *C. edule* post-settlement mortality due to predation (Jensen and Jensen, 1985; Whitton et al., 2012) and their ability to migrate, using a byssus thread to increase hydrodynamic drag, during this post-settlement period is understood to influence subsequent adult densities and spatial patterns (Armonies, 1994). However, interactions with other fauna may also be influential. Habitat engineering (Reise, 2002) through sediment surface disturbance by deposit feeders, burrowers and bioturbators may prevent larvae and post-larvae colonising affected areas, creating discrete patches in some species (Woodin, 1976). The importance of such interactions in soft sediments and the importance of the post-larval stage in *C. edule* for recruitment success (Beukema and Dekker, 2005), suggest that such processes could create spatial patterns in adult density.

Adults of the lugworm *Arenicola marina* (a burrowing deposit-feeding polychaete) and *C. edule* (a shallow burrowing surface-dwelling suspension-feeding mollusc) can make major contributions, 18.8 and 16.2 % respectively (Beukema, 1976), to the macrofaunal biomass in north western European intertidal flats (Beukema, 1976). They are both considered bioturbators because they modify the physical and biological characteristics of sediment, through the physical disturbance of their surroundings (Flach and de Bruin, 1994; Flach, 1996). This bioturbation occurs when lugworm cause surface sediment to enter their burrow at the feeding depression, and deposit sediment onto the sediment surface when defecating. When lugworms are excluded from an area the biological and physical habitat is likely to change. For example abundances in microbes can increase (Lei et al., 2010), meiofauna have been shown to decrease (Reise, 1985), and some macrofauna species (Flach, 1992; Volkenborn et al., 2009) and halophytic plants (Van Wesenbeeck et al., 2007) that depend on more stable sediment appear or increase in density. These biological responses are often due to the loss of the feeding burrows affecting oxygen penetration and the associated sediment reworking (Reise, 1985; Wendelboe et al., 2013). Although a less active

bioturbator, *C. edule* also disturbs the surrounding sediment when closing its shell valves, and when making horizontal and vertical movements (Flach, 1996).

Habitat modification by cockles and lugworm may influence the suitability of areas for *C. edule* post-larval settlement. Lugworm can reduce densities of post-larval *C. edule* when excluded from sediment (Flach, 2003, 1992). Similarly exclusion of *C. edule* has been shown to modify post-larval density (Flach, 2003, 1996), but outcomes have been variable. These studies found no effect or even an increase in post-larval density (de Montaudouin and Bachelet, 1996; Van Colen et al., 2013), temporally variable outcomes (Andre and Rosenberg, 1991) and in general there is little evidence of a negative linear relationship between adult cockle density and post-larval density. Interestingly, recent studies have demonstrated that adult cockles may facilitate settlement success through sediment stabilisation under certain conditions (Donadi et al., 2014, 2013b), and so uncertainty remains about the direction and predictability of adult / post-larval interactions in cockles.

Interactions between macrofauna and *C. edule* post-larvae may be operating directly or indirectly. Direct interactions may include ingestion of the post-larvae by macrofauna (Hiddink et al., 2002b), and mortality or emigration as a consequence of disturbance caused by bioturbatory activities (Armonies, 1996). Indirectly the presence of macrofauna may increase post-larval mortality through predation, by increasing their vulnerability as they are disturbed and become exposed at the sediment surface or through increasing their movement (Flach and de Bruin, 1994; Flach, 2003).

The overarching question of this study was what effect macrofauna are having on *C. edule* post-larval densities. To address the question combined field and laboratory experiments were conducted. The field experiment conducted tested the effect of macrofauna on natural settlement and post-settlement densities, with the laboratory studies seeking to investigate the poorly understood causative processes. In the field, we tested the hypothesis that the presence of *A. marina* and adult *C. edule* independently decrease post-larval density compared to lugworm and cockle free areas. It is expected that the predicted effect will increase in magnitude over time due to increasing interaction events with macrofauna. In the laboratory experiments were established to assess both direct effects of adult *A. marina* (ingestion, smothering and disturbance due to feeding activities) and adult *C. edule* (smothering and disturbance due to the movements of adult *C. edule) and the role of refugia in modifying disturbance effects.* In addition using a flume, we investigated whether the presence of macrofauna increased the number of *C. edule* post-larvae undertaking byssus drifting compared with those in macrofauna free sediment. Byssus drifting has a high potential to create spatial patterns and density changes post-settlement. If certain conditions that vary in space and time trigger migrations, such as macrofauna presence, then spatial patterns could change significantly over a few tidal cycles (Armonies, 1994).

2. Methods

2.1 Field experiment general approach

To observe the possible effects of adult cockles *C. edule* and lugworm *A. marina* on colonisation of *C. edule* post-larvae, separate field experiments were undertaken in the Dee estuary, on the English-Welsh border (53°20'52 N, 03°10'38 W) (Fig. 1A), and Traeth Melynog on the Isle of Anglesey, North Wales (53°08'22 N, 04°19'47 W) (Fig. 1B). In the Dee estuary cockles were excluded from experimental plots and at Traeth Melynog lugworm were excluded, as each location was best suited to manipulating only one of the macrofauna species. 18 plots were established at each location comprising a 3 x 6 grid of 1 m² plots, with a 10 metre separation between each plot (Fig. 1). Three treatments were randomly allocated across the 18 plots at each site: 1) an untouched control 2) an exclusion treatment where either lugworm or adult cockles were excluded and 3) a procedural control to account for the disturbance of excluding these macrofauna. The corners of each plot were marked with a bamboo stake that extended less than 10 cm above the sediment, to minimise drag and the chance of algae accumulating on the markers. The plots were sampled following the first detection of larval settlement (May to June) for 3 to 4 months, to understand how densities of post-larval cockles changed temporally among the treatments. *C. edule* post-larvae were quantitatively sampled using a 64 mm diameter corer (3217 mm²) to a depth of 15 mm. Random replicate cores were taken from each plot and the number of post-larvae counted and pooled.

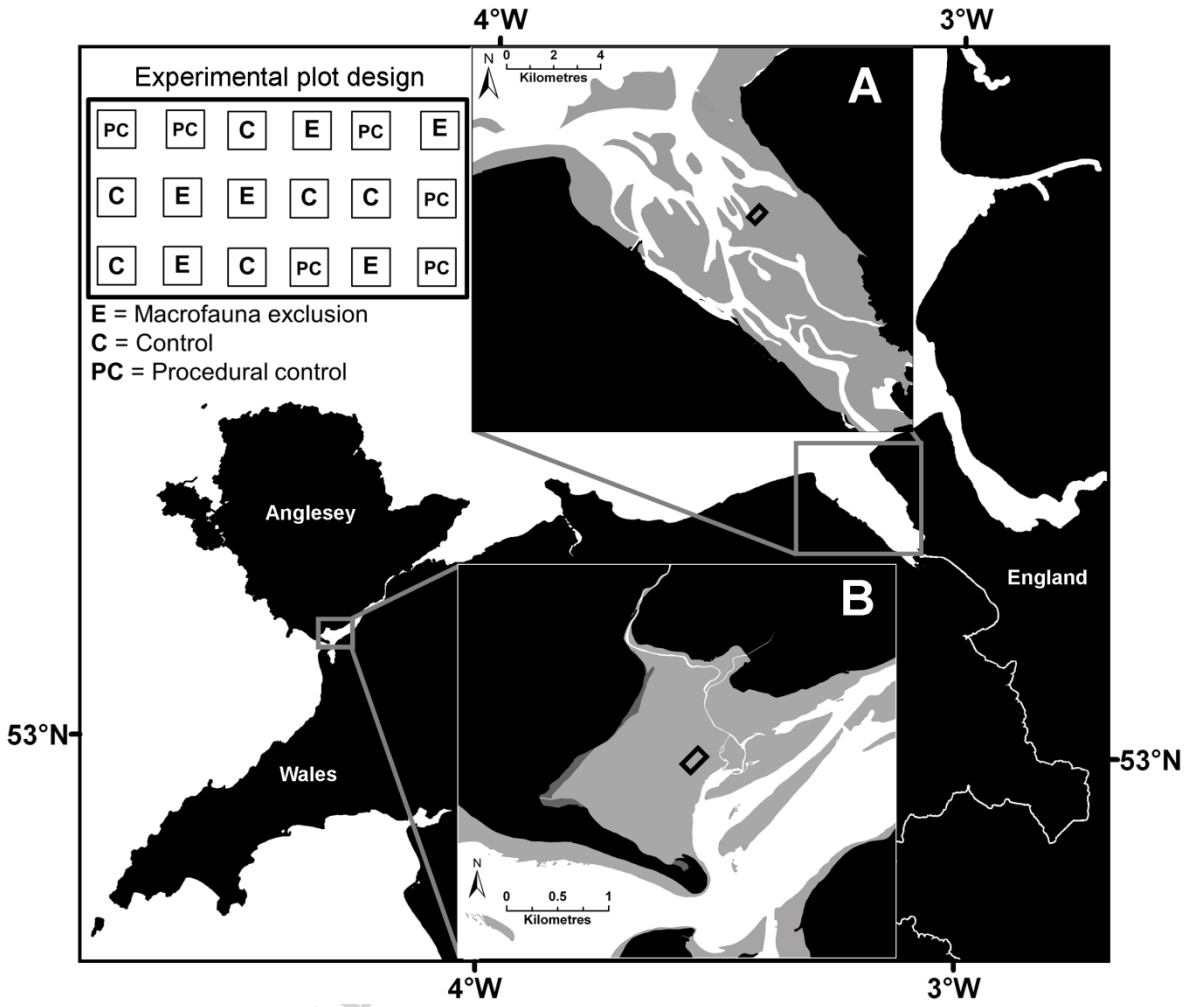


Figure 1. Design and location of the adult *Cerastoderma edule* exclusion experiments in the Dee estuary (A) and *Arenicola marina* exclusion experiment at Traeth Melynog (B), UK. Experimental plot locations shown by the black rectangle with grey areas showing the intertidal sediment and black areas the terrestrial land above MHWS. Experimental plot design insert is not to scale.

2.1.2 Adult C. edule exclusion field experiment, Dee estuary

Individuals of *C. edule* over 15 mm in length were removed from the 1m^2 experimental exclusion plots on 9th May 2012. Plots were raked to a depth of 15 cm and the sediment placed into a mesh bag. This allowed the sediment to pass through but retained cockles. The procedural control plots were raked in the same way, but instead of sieving the sediment the cockles that were retained by the rake were redistributed in the plot and allowed to re-burrow. Control plots were left undisturbed. Underlying patchiness in standing water pools and microphytobenthos amongst the plots was observed, but not quantified. Plots were sampled on three occasions using three pooled cores per plot (< 1 % of total plot area) on 17th May, 19th June and the 2nd July 2012. Sampling was not conducted after 2nd of July because this marked the beginning of the cockle fishing season in the Dee estuary,

when the chance of human disturbance to experimental plots was high. On the final sampling occasion sediment from a 0.1 m² quadrat was removed to a depth of 5 cm in the centre of each plot and sieved over 2 mm mesh to ascertain if there were any large cockles (>2mm) in the experimental plots that had either escaped the initial raking procedure, or migrated into the plots afterwards.

2.1.1 Lugworm exclusion field experiment, Traeth Melynog

Lugworm in the experimental plots were excluded by digging out the sediment in the plots to a depth of at least 10 cm. Subsequent immigration of lugworms into the plots was prevented by burying a horizontal 1 m² square of plastic mesh (2 x 2 mm mesh) at a depth of 10 cm in the sediment, and returning the excavated sediment onto the mesh (Fig. 2). This method has successfully been used in other studies to exclude lugworm from sediment (Volkenborn and Reise, 2007, See 2006; Volkenborn et al., 2009). The procedural control plots were created by digging and turning over the sediment to a depth of 10 cm in the same way as the exclusion plots, but without any mesh being placed before the sediment was returned. Plots were dug on 18th April 2012 to allow enough time (29 days) for recovery of the sediment structure from disturbance before sampling for *C. edule* post-larvae on 16th May, 18th June, 20th July, 26th July and 23rd of August 2012. During the first three sampling events three cores were taken per plot and pooled, but from July to August eight cores were taken per plot due to decreasing densities of post-larvae. On the 20th July the number of lugworm feeding depressions and faecal casts present within three 0.1 m² quadrats per plot were recorded. Build-up of loose macro-algae around the plot markers was removed on each sampling occasion from both the Dee estuary and Traeth Melynog locations (Fig. 2A and B).

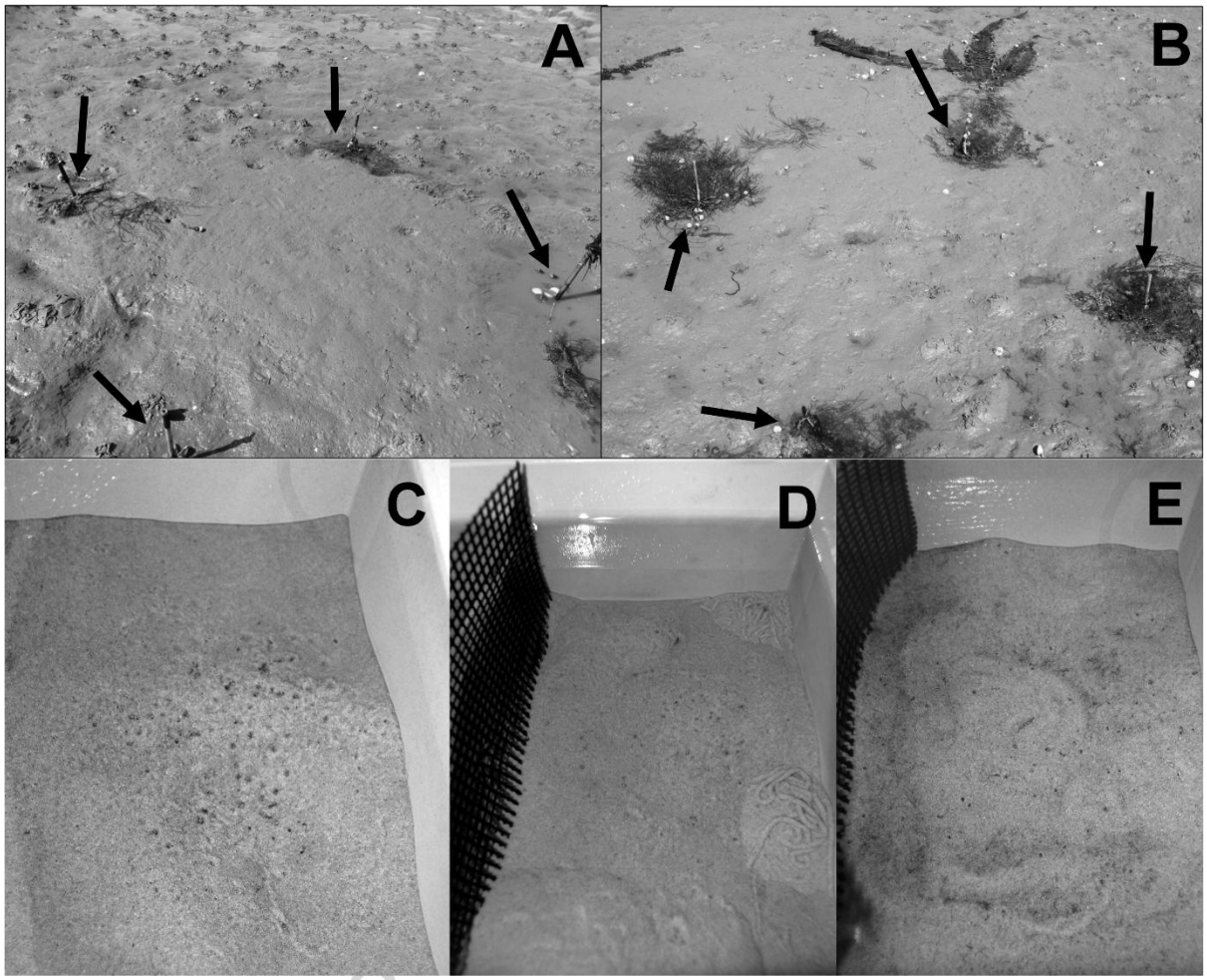


Figure 2. Examples of macrofauna disturbance in field (A and B) and laboratory experiments (C, D and E). Lugworm *Arenicola marina* experimental plots illustrating that when lugworms were excluded from the 1 m² plots the sediment surface became more homogeneous (A), compared to control plots where lugworm feeding depressions and faecal casts remained (B). Loose macroalgae accumulated on the bamboo stakes marking the plot corners (shown by arrows). Examples of the sediment surface in control without macrofauna (C), lugworm *A. marina* (D) and adult cockle *Cerastoderma edule* (E) experimental aquaria. Increased sediment heterogeneity can be seen in lugworm (D) and adult cockle (E) treatments.

2.2. *Laboratory experiments*

2.2.1. Aquarium study

2.2.1.1 Macrofauna presence and density

To determine the effect of macrofauna identity and density on post-larval cockle survival and growth, a two-way factorial design was used. The factor 'macrofauna' had three levels: adult cockles present; lugworms present and a control with no macrofauna. The second factor was 'density', with two levels: high and low macrofauna density (the control treatment of 4 replicates was conducted twice resulting in a fully balanced design of 6 treatments in total, high factor level represented densities of 143.6 m^{-2} and 41.0 m^{-2} and low factor level 71.8 m^{-2} and 20.5 m^{-2} for cockles and lugworms respectively) (Fig. 2). A significant effect of

'macrofauna' on cockle post-larval survival was expected, due to predicted lower survival with lugworm compared to adult cockles, with control treatments predicted to have the highest survival. The densities of macrofauna used were comparable with those found naturally in the field locations (~150 m^{-2} for adult cockles and 25 m^{-2} for lugworms observed in the field). Each treatment had four replicates (24 aquaria in total across all treatments) simultaneously conducted in plastic aquaria measuring 32.5 x 30 x 40 cm (length x width x depth) in size. The aquaria were closed systems supplied with aeration, and 50% water changes every two days were conducted using filtered seawater (settled and passed through 45 µm mesh). Each aquarium was filled with kiln dried sand to a depth of 24 cm to allow the lugworms sufficient depth to burrow. The sediment was sieved through a 500 µm mesh and the fraction >500 µm removed, to facilitate easier sampling of the post-larvae at the end of experiment, as post-larvae would be > 500µm in shell length. Rigid plastic mesh (5 x 5 mm mesh size) was placed into the sediment from the bottom of the aquarium to protruding above the water surface in all treatments to maintain macrofauna distribution across the tank area. This mesh divider only enabled cockle post-larvae to move through it.

Adult cockles and lugworm used in the experiment had mean lengths of 29.0 ± 0.4 and 131.6 ± 4.3 mm (±95% CI) respectively. Adult cockles and lugworm were added to the aquaria one week before the *C. edule* post-larvae to allow acclimation, and to allow time for elevated crawling activity to become reduced (Richardson et al., 1993). Two hundred post-larvae were added to each aquarium with one hundred haphazardly distributed on each side of the dividing mesh. The initial average density of *C. edule* post-larvae corresponded to 2,254 m^{-2} which is at the upper end of the natural density at Traeth Melynog, and at the lower end of cockle post-larval density measured in the Dee estuary soon after settlement (Whitton et al., 2015). Post-larval cockles were collected from the Dee estuary a month before the experiment began on the 18th July 2012. A representative sample of 50 post-larvae were measured using a calibrated eyepiece giving a mean length of 1.8 ± 0.7 mm (± 95% CI) at the beginning of the experiment so growth could be measured at the end of eth experiment. Each aquaria was dosed three times a day with the microalgae *Isochrysis galbana* (T-Iso) to achieve an algal concentration of ~1.3 x 10^9 cells tank $^{-1}$ (Helm et al., 2004). The supplements of microalgae were used to maintain macrofauna, (through suspension feeding or deposit feeding on settled microalgae), and prevent food being a limiting factor for growth and survival of post larvae during the two-week experiment. Temperature and salinity were measured daily (mean seawater temperature 17.7 °C, salinity 32). Upon completion of the experiment *C. edule* post-larvae were recovered by siphoning off all the sediment from each half of the tank separately over a 500 µm sieve. Recovered post-larvae were counted, and shell length measured in a sub-sample from each aquarium.

2.2.1.2 Refuge provision

To test the effect of availability of refugia from macrofaunal disturbance on post-larvae a similar experimental approach as above was used. This experiment recognises that natural distribution of macrofauna is not homogenous and that patchiness in distribution will result in spatial refugia. Thus, the effect of macrofauna (7 adult cockles and 2 lugworms representing 71.8 m^{-2} and 20.5 m^{-2} respectively) distributed across the mesocosm (no refugia) was compared with a treatment where the same number of macrofauna were only placed on one side of a dividing rigid plastic mesh. This mesh was placed vertically in the sediment from the bottom of the aquarium to protruding above the water surface (refuge provided) (Fig. 2). The refuge will potentially allow post-larvae to crawl away from macrofauna and the associated disturbance, and thereby increase survival and growth. Presence / absence of refugia was implemented using different identities of macroafauna (adult cockles x presence / absence of refugia; adult lugworms x Presence / absence of refugia; and a control treatment with no macrofauna conducted twice to ensure a fully balanced design). Thus, a two-way factorial design was implemented with 6 treatments (four replicates per treatment) in total. At the end of the experiment, the number of dead and live post-larvae on both sides of mesh divider was recorded to determine any refuge use.

2.2.2. Flume study

To assess the effect of macrofauna disturbance on drifting behaviour of post-larvae, the number of byssus-drifting *C. edule* post-larvae was compared in the presence and absence of macrofauna using a laboratory flume (2 m long clear acrylic flume with a square working cross sectional area of 169 cm^2, containing 150 L of re-circulating seawater pumped by a 12 Volt motor driven propeller). On the floor in the middle of the flume a sediment box (24 x 11.5 x 25 cm, length x width x depth) filled with 2 parts < 500 µm kiln dried sand and 1 part dried mud collected from the Dee estuary contained post-larval cockles and macrofauna. The sediment in the box was flush with the floor of the flume working area, whilst the remainder of the flume floor was covered with a thin layer of the same sediment to ensure equivalent bed roughness across the entire flume working area. A 500 µm net covered the flume's complete cross section downstream of the sediment box to collect any drifting *C. edule* post-larvae. The three treatments (adult cockles present, lugworm present and a control with no macrofauna) were replicated five times. A flow of 3 cm sec^{-1} (measured by an ultrasonic doppler velocity profiler at the sediment surface) was found to be the most suitable current flow to initiate byssal drifting without scouring of the sediment surface. This current speed is typical of the observed speeds either side of slack water on intertidal flats (Collins et al., 1998).

At the start of an experiment, the flume was filled to capacity with 40 µm filtered seawater. Three adult cockles (range 30 - 35 mm) or 1 lugworm (90 - 150 mm), achieving a density similar to that in the laboratory experiments and in the field (i.e. adult cockles ~150 m^{-2} and lugworm 25 m^{-2}), were placed in the sediment box and allowed to burrow. At the start of each experimental run the circulating pump in the flume was switched on, providing a flow rate of 1 cm sec^{-1}, for 6 hours and then switched off. Eighty post-larval *C. edule* collected from the Dee estuary with a mean shell length ± 95% CI of 1.7 ± 0.02 mm were then randomly added to the sediment box and allowed to burrow. Two hours later the circulating pump was switched on again, initially generating a current flow of 0.5 cm sec^{-1} that was then increased incrementally until 3 cm sec^{-1} was attained after 1 hour. *Isochrysis galbana* (T-Iso) cells were added to achieve an initial concentration in the flume of 1.75 x 10^9 cells L^{-1}. The flume was covered to exclude ambient light as byssus drifting has been shown to occur more in darkness (Armonies, 1992; Hiddink et al., 2002a). After a further six hours the pump was switched off, the net removed from the flume, and any post-larvae retained were counted and measured. At the end of each experiment all sediment in the sediment box and flume were siphoned out and passed over a 500 µm mesh. All retained *C. edule* post-larvae were counted and a sub-sample from each experiment measured for shell length, to test for differences in the size of drifters and those remaining in the sediment.

2.3. Data analysis

Data were checked for deviations from normality and equal variance using Kolmogorov-Smirnov and Levene's test respectively before analysis of variance (ANOVA) was conducted. Where the assumptions of ANOVA were not met, data were $\log_{10}$ transformed. If assumptions were still not met the non-parametric Kruskal-Wallis test was used. The field experiment plots for each treatment were repeatedly sampled over time and therefore a repeated measures (mixed-effects) ANOVA was used, as each sampling time could not be strictly considered as independent. Sphericity was tested using Mauchly's test before the repeated measure ANOVA was conducted. Sphericity was not met for the lugworm plot data, and so Greenhouse-Geisser adjusted degrees of freedom were used. The assumption of homogeneity of variance could not be met for shell length data in the aquarium study when conducting the factorial ANOVA, even after outliers were removed, hence differences for each level of the factor 'density and refuge' were analysed using separate one-way ANOVAs for each level of the 'macrofauna' factor. All means presented ± 95% CI unless stated otherwise.

3. Results

3.1 Field exclusion experiments

3.1.1 Adult C. edule exclusion experiment

Adult *C. edule* exclusion plots did not completely remove all cockles but resulted in significantly lower adult *C. edule* densities (mean density 17 ± 18 m^{-2}; $F_{(2,15)}$ = 7.87, p = 0.005, (x+1) $\log_{10}$ transformed data) and with smaller variability in numbers than the control and procedural plots (Fig. 3A). The control and procedural plots had a mean adult *C. edule* density of 105 ± 92.6 and 160 ± 138.8 m^{-2} respectively, and were not significantly different from each other (Tukey post-hoc comparison p = 0.019).

On the first sampling event of May 5th all *C. edule* post-larvae were <750 µm in shell length with >70 % <500 µm, indicating that larval settlement was occurring at this time. On the last sampling event on the 2nd of July the mean shell length was 2733 ± 118 µm. *C. edule* post-larval densities were highest in the control plots with slightly lower densities in the procedural control plots (Fig. 4A). Density of post-larvae in the adult *C. edule* exclusion plots were significantly lower than control and procedural control plots (Repeated measures ANOVA on $\log_{10}$ transformed data, between-subjects factor, $F_{(2,15)}$ = 4.634, p = 0.027) (Fig. 4A). On average across the three sampling events the densities of post-larvae in the exclusion plots were 50% lower than those in the procedural control (ranging from 59% lower in May to 44% lower in June). Densities of *C. edule* post-larvae in plots showed a consistent effect of the treatment over all sampling dates (Fig. 4A) (non-significant interaction, $F_{(4, 30)}$ = 0.608, p = 0.66). Density of post-larvae significantly increased with time (within-subjects factor, $F_{(2, 30)}$ = 87.678, p <0.001) during the experiment, indicating net migration into plots between June and July as no settlers (< 500 µm) were found during this period.

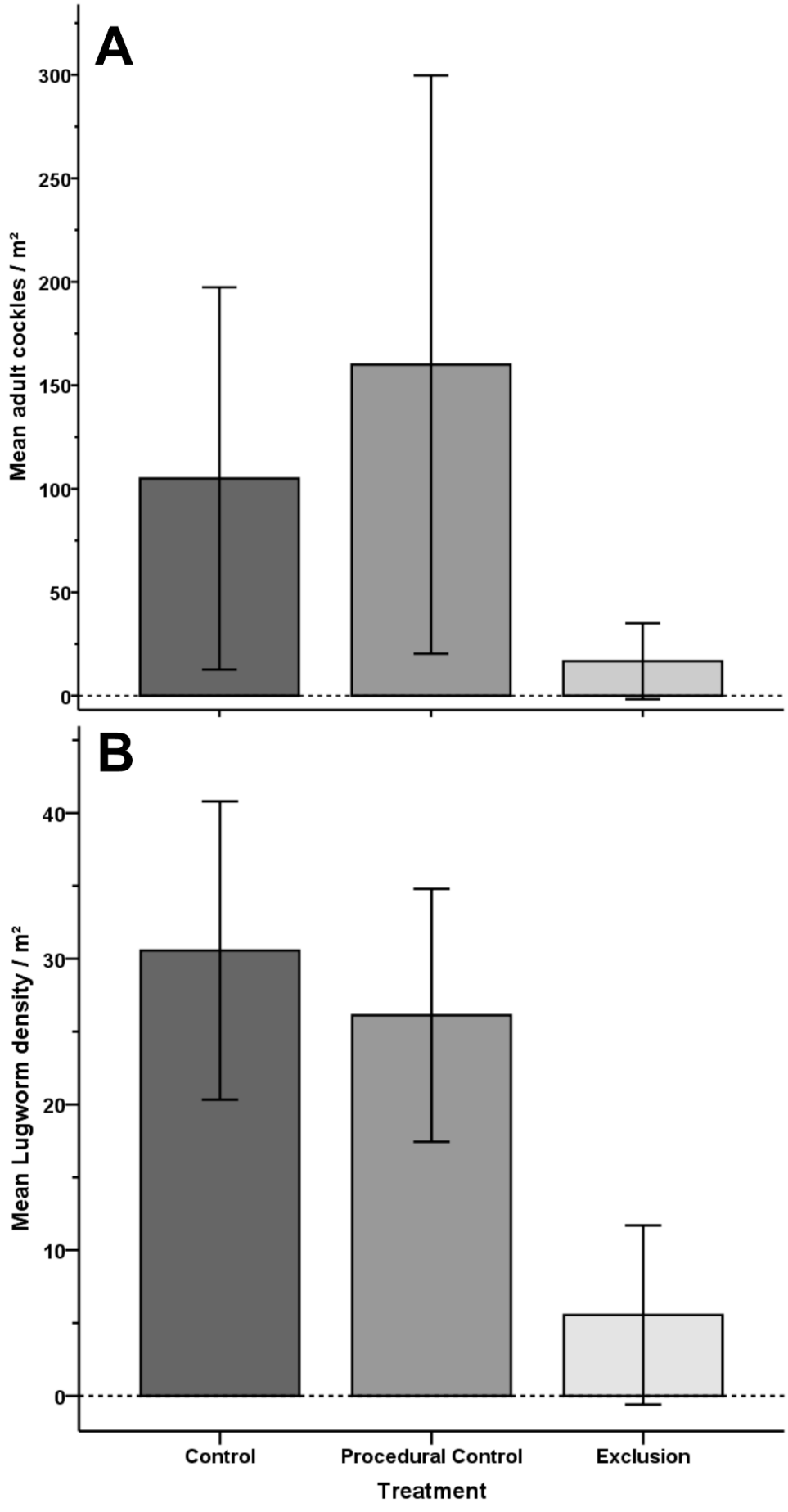


Figure 3. Mean density of adult cockles *Cerastoderma edule* (A) and lugworm *Arenicola marina* (B) for each field experiment treatment. Error bars show the 95% confidence interval for the mean and n = 6 per treatment.

3.1.2 Lugworm exclusion experiment

Some lugworms were found in the exclusion plots after the experimental manipulation. However the mean density of lugworm was < 20% of the control and procedural control plots (Exclusion 5.6 ± 6.2, Control 30.6 ± 10.3 and Procedural Control 26.1 ± 8.7 m^{-2}, $F_{(2,17)}$ = *16.20, p* = <0.001) (Fig. 3B). Lugworm density in the control and procedural control plots were not significantly different (Tukey post-hoc test, p = 0.619).

On the first sampling occasion 93% of the *C. edule* were <1000 µm in shell length, indicating larval settlement had occurred recently, and the presence of individuals of ≤400 µm in shell length indicated settlement was still underway. Small post-larvae with a shell length <1000 µm were found on all sampling occasions. This suggests larvae were likely settling beyond the main settlement period in May and continually colonising the plots.

The post-larval density was significantly higher in the lugworm exclusion plots than the control and procedural control plots during the experiment (between-subjects factor, $F_{(2,15)}$ = 4.868, p = 0.023), with the exception of the first sampling event during the main settlement period (Fig. 4B). The mean density of *C. edule* post-larvae in the lugworm exclusion plots was approximately double or more than that measured in the procedural control plots, again with the exception of the May 16th sampling event. Repeated measures ANOVA also indicated a significant effect of sampling date on post-larval density (within-subject factor, $F_{(1.8,\ 27.1)}$ = 13.891, p <0.001) as expected due to density changes over time. The control and procedural control plots had similar densities throughout the experiment giving confidence in the validity of the lugworm exclusion effect on post-larval densities (Repeated measures ANOVA on control and procedural control only, $F_{(1,10)}$ = 0.740, p = 0.410). No interaction between factors was found ($F_{(3.62,27.14)}$ = 0.848, p = 0.498).

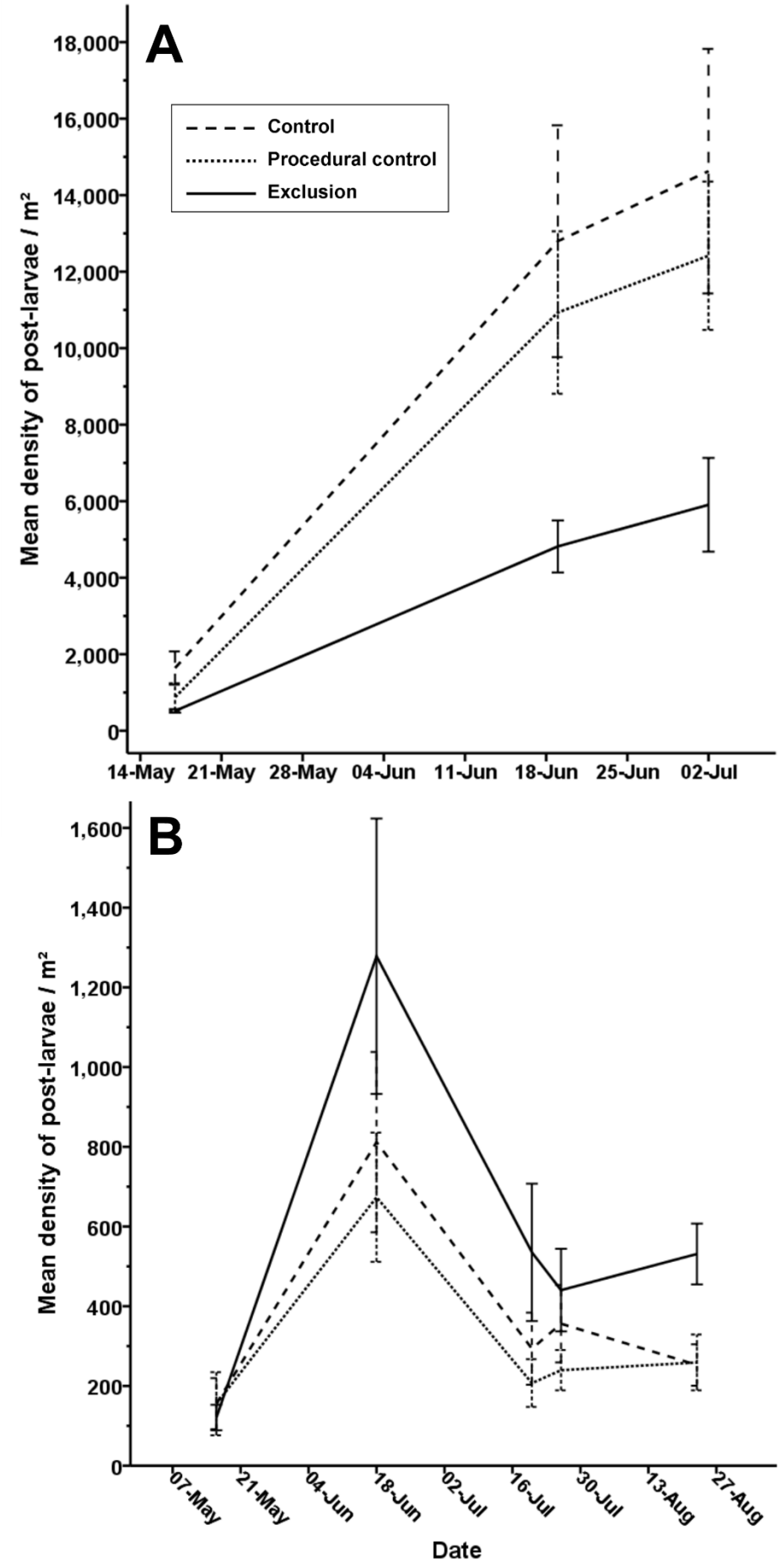


Figure 4. Mean density of cockle *Cerastoderma edule* post-larvae in the adult cockle (A) and lugworm (B) exclusion field experiments. Error bars show one standard error ± the mean (n = 6 per data point).

3.2 Laboratory studies

3.2.1 Aquarium study

3.2.1.1 Survival of C. edule post-larvae in the presence and absence of macrofauna

No significant effect of macrofauna presence (arcsine transformed proportion, $F_{(1,18)} = 1.7$, $p = 0.211$) or density ($F_{(1,18)} = 0.02$, $p = 0.889$) on post-larval survival was observed on post-larval survival (Fig. 5A). However, there was a trend for lower survival (and higher variability) in the control treatments (no macrofauna), and treatments with lugworms present compared to those with adult cockles present (Fig. 5A). The upper limit of survival for each treatment was between 91 and 97% survival, with most above 85% survival. Five replicates had noticeably lower survival of below 70% (3 from lugworm treatments and 2 from control treatments), one of which from a control replicate was below 50%. This indicates that some of the replicates resulted in high post-larval mortality independent of macrofauna presence.

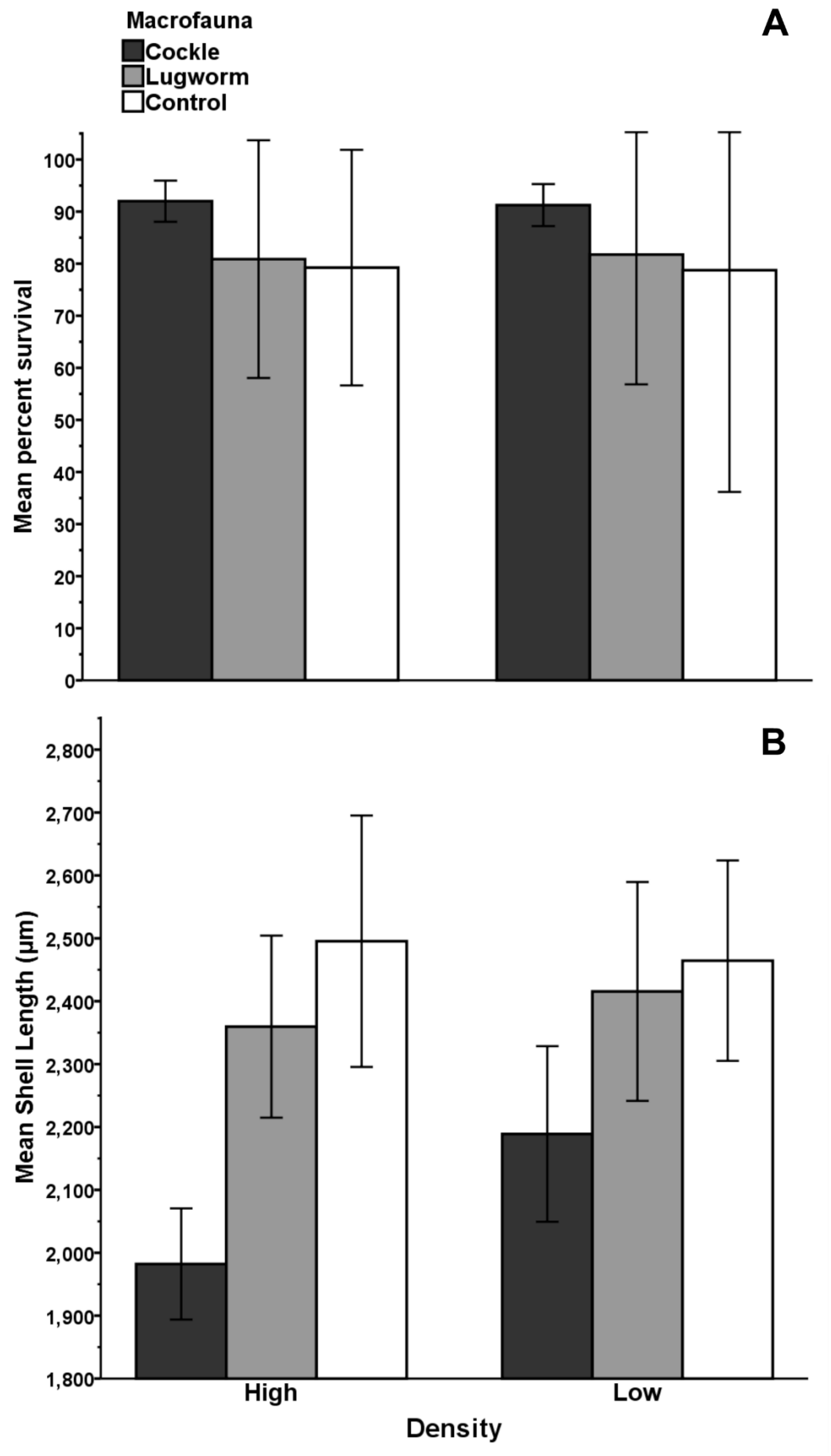


Figure 5. Mean survival (A) and shell length (B) of post-larval cockles *Cerastoderma edule* when confined with adult cockles, lugworms *Arenicola marina* and control treatments (which had no macrofauna) across levels of high and low macrofauna density. Origin of y-axis in B represents mean shell length at the start of the experiment. Error bars show 95% confidence interval for the mean (n = 4 per treatment).

3.2.1.2. Growth of Cerastoderma edule post-larvae

The mean shell length of *C. edule* post-larvae at the beginning of the experiment was 1801 ± 66 µm (1.8 ± 0.6 mm). Shell lengths at the end of the experiment (Fig. 5B) showed significant differences (Kruskal Wallis test, χ^2= 28.66, p= < 0.01). Significant pairwise comparisons suggest this to be driven by the lower final shell length in the high density adult cockle treatment (mean 2 mm) when compared to the higher growth in the lugworm treatments (mean 2.5 mm, p = 0.001 for both lugworm treatments) and a control treatment (mean 2.4 mm, p = 0.001) (Fig. 5B).

3.2.1.3. Use of a spatial refuge

Two of the four lugworm refuge treatments failed due to lugworms managing to get past the mesh divider and so disturbing both sides of the aquaria, therefore could not be tested for refuge use. For the adult cockle treatments there is no evidence that refuge provision affected post-larval survival (Two-way ANOVA on arcsine proportion survival, 'refuge provision' factor: $F_{(1,12)}$ = 0.297, p = 0.596, 'macrofauna presence' factor: $F_{(1,12)}$ = 1.231, p = 0.289). In addition there is no evidence that fewer dead, or more live cockles, were recovered from the macrofauna free refuge side (One-way ANOVA, $F_{(1,6)}$ = 0.798, p = 0.406 and $F_{(1,6)}$ = 0.002, p = 0.964 respectively). No significant difference (One-way ANOVA, $F_{(1,88)}$ = 0.367, p = 0.546) in final shell length was found between post-larvae with 'refuge provided' compared to those with 'no refuge'.

3.2.2. Flume study

The number of post-larvae captured in the net was not significantly different between treatments (arcsine transformed proportion data, one-way ANOVA, $F_{(2,12)}$= 0.743, p = 0.496) (Fig. 6). Observations during the experiment indicated that in three out of the five lugworm treatments there was no evidence of feeding or bioturbation (no feeding funnel or faecal casts). Post-larvae quickly burrowed into the sediment after being added, but at the end of the experiment some post-larvae could be observed on the surface, with a byssus thread trailing behind (Fig. 7). The number of post-larvae drifting was low in all treatments, and reached a maximum of 33 out of the 80 post-larvae added (41.3%) in one of the adult cockle replicates. The size distribution of post-larvae drifting did not differ between treatments. Post-larvae recovered from the net had a smaller size (lower minimum, maximum and

median shell length) than those recovered from the sediment box across all treatments (Median length, Kruskal Wallis test, χ^2= 13.6, *p*= < 0.001).

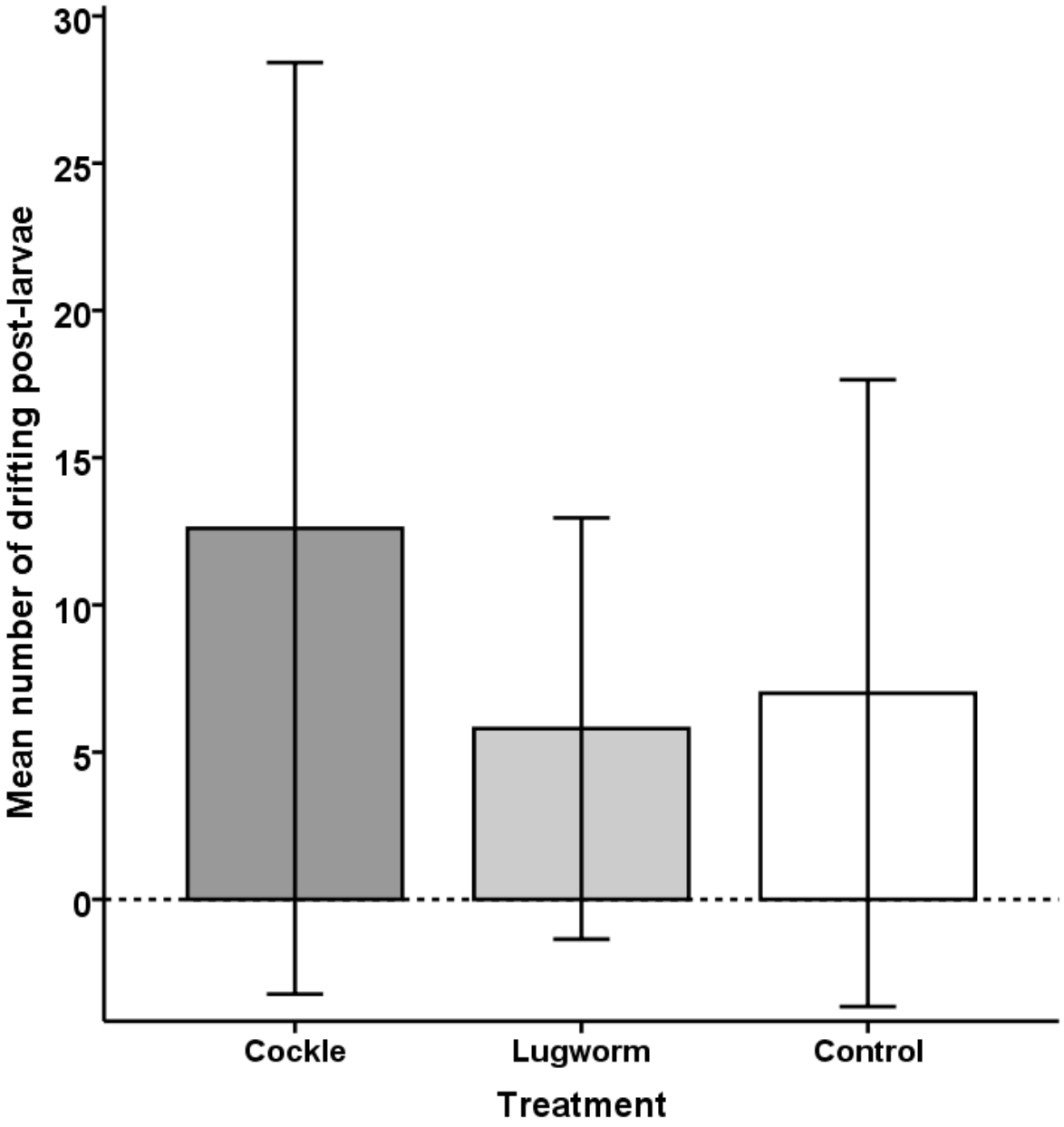


Figure 6. Number of *Cerastoderma edule* post-larvae drifting during the flume experiment with macrofauna (Adult cockle *C. edule* and the lugworm *Arenicola marina*) present in the sediment and devoid of macrofauna (Control). Error bars are 95% confidence intervals.

Figure 7. A post-larval *Cerastoderma edule* (circled) from the flume experiment with sand grains attached to it's single byssus thread (the byssus thread is being held by forceps at the top of the picture).

4. Discussion

The presence of macrofauna changed the settlement success of cockle post-larvae. Lugworm presence depressed settlement success by half; in contrast the presence of adult cockles significantly increased the settlement success of cockle post-larvae. The growing evidence of a positive feedback of adult cockles on post-larval densities has new and important implications of our understanding of *C. edule* ecology and its fishery and conservation management. The aquarium experiments require validation, but may suggest that direct interactions with macrofauna from ingestion, bioturbation or increased emigration by byssus drifting may not be the causal processes driving our field experiment results.

Previous studies on soft sediment infauna have shown that the exclusion of lugworms can have contrasting outcomes on different species (Reise, 2002). Our field experiment shows clearly that *C. edule* post-larvae benefit from lugworm exclusion. *C. edule* post-larvae gain little benefit from the positive effects of lugworm presence that are experienced by some species, such as increased local oxygen penetration into the sediment and increased bacterial production. This is because cockle post-larvae are suspension feeders and shallow burrowers that occupy the top few mm of sediment, which is sufficiently oxygenated by diffusion. Stable sediment is likely a desirable feature for *C. edule* post-larvae, enabling feeding at or near the sediment surface and avoiding the risk of being smothered by lugworm faecal casts or falling into the feeding depression and associated shaft. The reduction in levels of disturbance that occur following lugworm exclusion often

benefit species which prefer stable sediment, such as the tube building polychaetes *Pygospio elegans* and *Lanice concheliga* (Donadi et al., 2015; Volkenborn et al., 2009). The presence of lugworms has been shown to result in a decrease in observed numbers of *C. edule* post-larvae on small spatial scales, on average by 52%, in other field experiments (Flach 1992, 2003). However there does not appear to be a linear relationship of lugworm density and post-larval density (Flach 1992). From past studies and ours there is strong evidence that the presence of lugworms will reduce post-larval densities by around 50% when compared to adjacent lugworm free sediment, but this can be context dependant (Donadi et al., 2014).

On the first sampling event at the initial settlement period lugworms did not depress post-larval numbers, and adult cockles had a smaller positive effect. This suggests that at small scales (≤ 1 m²) the presence of lugworms and adult cockles has a different or no effect on initial settlement, despite being the period when post-larvae are at their smallest and therefore potentially most vulnerable (Andre and Rosenberg, 1991). In agreement with Flach (2003, 1996) we suggest that a interactions with macrofauna change post-larval densities during the post-settlement period, rather than at settlement itself.

In previous studies using adult cockle densities of 55 – 70 m^{-2} had either a positive or no effect on conspecific post-larval densities (Flach, 1996; Van Colen et al., 2013). In the same studies at higher densities of adult cockles a notable reduction was seen in post-larval density. This indicates there may be a threshold density of adult cockle presence determining if the interaction is unimportant, beneficial, or negative for post-larvae. This potential threshold density is likely context dependant. In general adult cockles are seen as bioturbators and have been shown to increase the erosion of sediment (Andersen et al., 2010; Ciutat et al., 2007). In contrast Donadi et al. (2013a) showed that dense areas of adult cockles can actually stabilise sediment and increase the microphytobenthos production, compared to cockle free or lugworm inhabited sediment. This sediment stabilisation is thought to be responsible for observed higher cockle juvenile (0-group) densities in areas with adult cockle additions (Donadi et al., 2014). Other hypotheses can be proposed, such as gregarious settlement with adult conspecifics and other intraspecific positive feedbacks. For example predation is a key driver of post-settlement mortality (Beukema and Dekker, 2005), and adult cockles could increase habitat complexity and therefore have the potential to reduce predator foraging efficiency (Grabowski et al., 2008). The many possible explanations necessitate further replication and experimental work to identify the causative processes of these field experiments and observations.

After the two week aquarium studies there was no evidence of increased mortality through direct ingestion or sediment smothering, with high survival in most experimental tanks across the treatments. The low survival in some replicates, including the control

treatment, may be attributed to poor water quality due to uneaten microalgae. This is suggested due to comparisons with the adult cockle treatments, where significant filter feeding by the adult cockles was occurring and variability in post-larval survival and growth was much lower. A previous preliminary study conducted that confined *C. edule* post-larvae with lugworms in aquaria found no difference in mortality with control lugworm free aquaria (Leedham, 2011). However, there was an indication that movement of *C. edule* post-larvae increased in the presence of lugworms (Leedham, 2011). Observation of *C. edule* post-larvae show that they were highly active and able crawlers when disturbed, and it is likely that a post-larva may be able to return to the sediment surface if covered during lugworm defecation or adult cockle crawling activity. Adult bivalves and juvenile *Macoma* can vertically migrate to establish siphon surface contact and survive from a depth of 2 – 3 times their shell length (Chang and Levings, 1978; Hinchey et al., 2006; Maurer and Keck, 1981). When investigating mortality of post-larval *Macoma balthica* Hiddink et al. (2002b) found that the presence of lugworms did reduce post-larval abundance in plots and found post-larvae in *A. marina* stomachs. Laboratory feeding experiments did show that direct ingestion by *A. marina* of *M. balthica* post-larvae is possible (Hiddink et al., 2002b). Post-larvae of *M. balthica* have been shown to survive complete burial in anoxic sediment for up to 10 days (Elmgren et al., 1986), and Hiddink et al (2002b) concludes for *M. balthica* that direct mortality from sediment smothering caused by bioturbation is unlikely to be a significant cause mortality for post-larvae, as we do for *C. edule* post-larvae.

The flume study shows little evidence of differences in the number of cockle post-larvae drifting when in the presence of macrofauna. The drifting rates were generally low (1.3 – 11.3 %) but with some exceptions, particularly in the adult cockle treatment with one replicate having 41.3 % drifting. This experiment would merit further replication and refinement as the lugworms produced no, or very small, faecal casts which indicated little feeding activity was occurring, and so a poor representation of a natural bioturbation. A longer acclimation period and a higher sediment organic content may have stimulated more feeding and therefore bioturbation as seen in the wild. The flume study does not explain the differences seen in densities in the field study, but illustrates the potential for up to 40% of post-larvae to migrate in a short period of time and change densities post-settlement. Such changes in density therefore may not be attributed to macrofauna directly causing mortality. It is established that byssal drifting is an active behaviour in species such as *C. edule* and *M. balthica* (Armonies, 1994; Hiddink, 2002). Alternatively results of the field and flume experiments may have occurred passively, as lugworms and adult *C. edule* have been shown to modify the erodability of surrounding sediment, and therefore potential of post-larval resuspension compared to macrofauna free sediment (Andersen et al., 2010; Ciutat et al., 2007; Wendelboe et al., 2013). Our laboratory experiments to identify the causative

processes for field observations cannot be considered conclusive, with the causative process possibly having complex interactions and difficult to replicate in the laboratory.

4.1 Conclusions

Our study has demonstrated contrasting effects of the presence of lugworms and adult cockles on the density of *C. edule* post-larvae during the post-settlement period. Exclusion of lugworms increased post-larval densities, whereas exclusion of adult cockles decreased the density of post-larvae. This illustrates along with the work of Donadi et al. (2015, 2014, 2013b) that the interaction between post-larvae and adult *C. edule* is not necessarily a negative one, and can be significantly positive. Depending on the scale at which this positive interaction occurs, it may have an important role to play in a population's recruitment success. Such an interaction potentially means that depleting an area of adults during cockle fishing, or any other activity, could reduce that areas' recruitment potential in subsequent settlement events by up to 50%. Recruitment success into cockle fisheries are largely dependent on post-larval survival up to the post-winter period, and so understanding the processes affecting numerical and spatial colonisation during the post-larval stage has important management and conservation implications. We have demonstrated that presence of macrofauna can effect post-larval densities and therefore recruitment success and spatial distribution, but the driving processes remain unclear from our laboratory experiments and warrant further investigation.

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